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Chemical Preparation Laboratory for IND Candidate Compounds

Annual Report

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January 31, 1989

(January 17, 1988 - January 16, 1989)

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16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
07	03		Nucleosides, triazoles, phenanthridones, alkaloids, antivirals, ribavirin, tiazofurin. RAI
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the reporting period twenty-one compounds were synthesized and submitted for testing Most of these compounds are derivatives of ribavirin or tiazofurin modified in their sugar or heterobase portion. One group of compounds are triazole heterobases with various substituents, while another group is derived from phenanthridone as the skeletal structure. A major effort was dedicated to the large-scale preparation of ribavirin amidine hydrochloride.

Compounds which remain under investigation are selenazole, thioformycin B, prodrug ester. and carbonitrile to amidine conversion studies. Keywords)

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I. SUMMARY

During the reporting period twenty-five target compounds have been examined, and the preparation of nineteen compounds was completed. Two of these compounds, AVS 4600 and AVS 4601, were synthesized twice and resubmitted.

The following compounds were delivered: 1,2,4-Triazole-3-carboxylic acid (AVS TCOOH); 1,2,4-Triazole-3-carboxamide (AVS TCA); 1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid (AVS RCOOH); Lycoricidine triacetate (AVS 360); 1-Hydroxy-2-acetoxy-lycoricidine (AVS 360MA); 1-Hydroxy-8,9-methylenedioxy-1,4,4a,106-tetrahydro-6(5H)phenanthridone (Intermediate II); 1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-methyl-imidale (AVS 4071); Protected and Deprotected tiazaofurin nitrile (AVS TFN); 1-(2',3'-Anhydro-β-*D*-ribofuranosyl)-1,2,4-triazole-3-carboxamide (AVS 4603); 3'-Deoxyribavirin (AVS 4602); 1-(2',3-Dideoxy-β-*D*-glycero-pent-2-enofuranosyl)-1,2,4-triazole-3-carboxamide (AVS 4600); 2',3'-Dideoxyribavirin (AVS 4601); 2',3'-Dideoxytiazofurin (AVS 4604); 2',3'-Dideoxy-2',3'-didehydrotiazofurin (AVS 4606); 2-(5'-Hydroxymethylfuran-2-yl)thiazole-4-carboxamide (AVS 4605); 4-Hydroxy-1,2,3-triazole-5-carboxamide (AVS 94); 1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (AVS 206); 9-β-*D*-Ribofuranosylpurine-6-thio-carboxamide (AVS 79).

The preparations of the following target compounds remains under investigation, and their syntheses are in progress: Thioformycin B (AVS 52); 1-β-*D*-Ribofuranosyl-4-hydroxy-1,2,3-triazole-5-carboxamide (AVS 136); Selenazole (AVS 253); 4-Carboxamidine-4-phenylpiperidine dihydrochloride (AVS PIP); Prodrug ester (AVS XYZ); 1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carbonitrile (AVS RCN);

II. FOREWORD

All information in this report is the property of the U.S. Army Medical Research and Development Command. The contractor retains no copyright or patent rights.

All target compounds reported herein were prepared in strict compliance with "Current Good Manufacturing Procedures" (CGMP) guidelines. All intermediates and final products unreported in the chemical literature were fully characterized by elemental and spectral analyses.

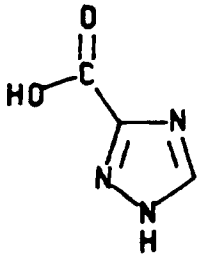
IIIa. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO U.S. ARMY MEDICAL RESARCH INSTITUTE OF INFECTIOUS DISEASES (USAMRIID) JANUARY 17, 1988 TO JANUARY 16, 1989

No.	Compound	Amount	Production Control No.
AVS TCOOH	1,2,4-triazole-3-carboxylic acid	4.0 g	2167
AVS TCA	1,2,4-triazole-3-carboxamide	4.0 g	2171
AVS RCOOH	1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid	4.7 g	2166
AVS 360	Lycoricidine triacetate	4.6 g	2212
AVS 360MA	1-Hydroxy-2-acetyl-lycoricidine	4.0 g	2125
AVS INT II	1-Hydroxy-8,9-methylene dioxy-1,4,4a, 106-tetrahydro-6(5H)-phenanthridone	2.08 g	
AVS 4071	1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-methylimidale	21.0 g	2294
AVS ATF	2(Tri-O-acetyl-β-*D*-ribofuranosyl) thiazole-4-carboxamide	7.7 g	2117
AVS TFN	2-β-*D*-Ribofuranosyl-thiazole-4-carboxamide	23.0 g	2117
AVS 4603	1-(2,3'-Anhydro-β-*D*-ribofuranosyl) -1,2,4-triazole-3-carboxamide	1.6 g	2198
AVS 4602	3'-Deoxyribavirin	1.1 g	2223
AVS 4600	1-(2.3-Dideoxy-β-*D*-glycero-pent-2 -enofuranosyl)1,2,4-triazole-3 -carboxamide	1.5 g 2.8 g	2138 2446
AVS 4601	2',3'-Dideoxyribavirin	1.1 g 2.3 g	2202 2452
AVS 4604	2',3'-Dideoxytiazofurin	1.7 g	2243
AVS 4606	2',3'-Dideoxy-2',3'-didehydro-tiazofurin	1.2 g	2232
AVS 4605	2-(5'-Hydroxymethylfuran-2-yl) thiazole-4-carboxamide	1.5 g	2218
AVS 94	4-Hydroxy-1,2,3-triazole-5-carboxamide	12.5 g	2316

IIIa. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO U.S. ARMY MEDICAL RESARCH INSTITUTE OF INFECTIOUS DISEASES (USAMRIID) JANUARY 17, 1988 TO JANUARY 16, 1989, Continued

No.	Compound	Amount	Production Control No.
AVS 206	1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride	1948 g	2553
AVS 79	9-β-D-Ribofuranosylpurine-6-thiocarboxamide	14.5 g	2549

IIIb. STRUCTURES OF COMPOUNDS SUBMITTED



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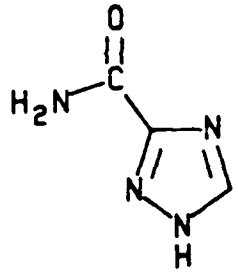

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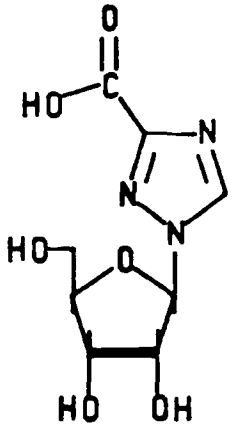

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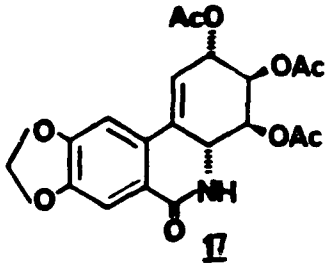

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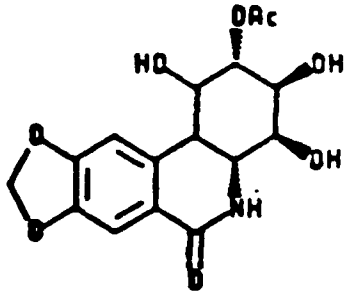

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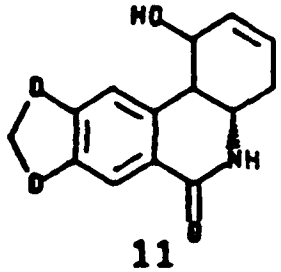

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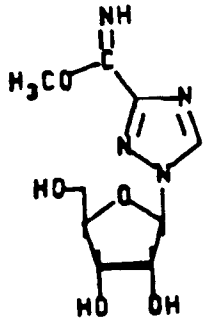

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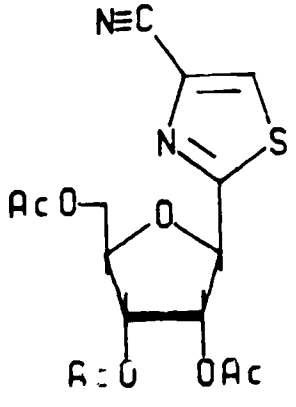
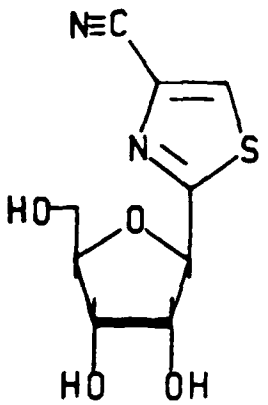


AVS ATF

IIIb. STRUCTURES OF COMPOUNDS SUBMITTED



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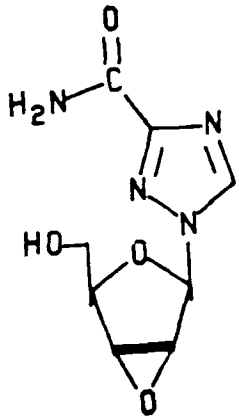

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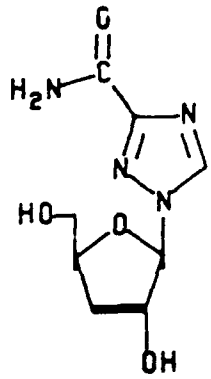

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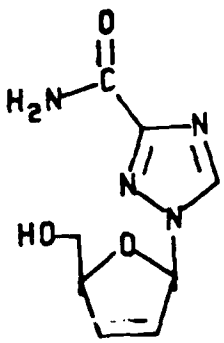

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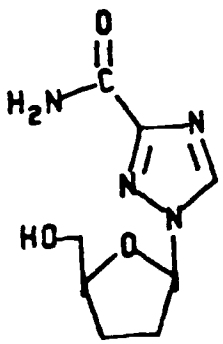

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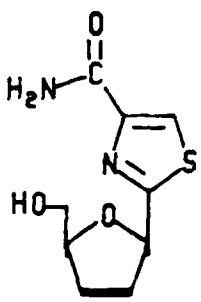

AVS 4604

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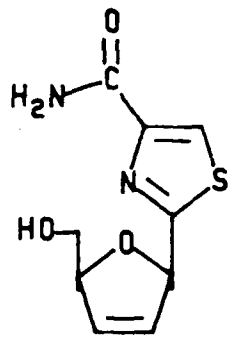

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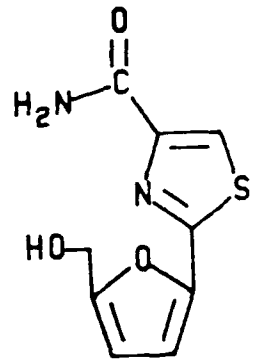

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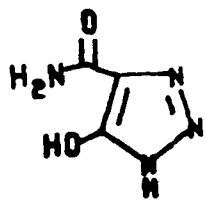

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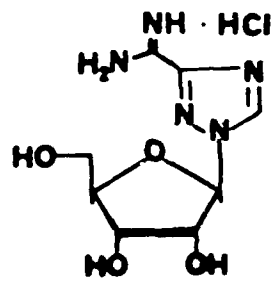

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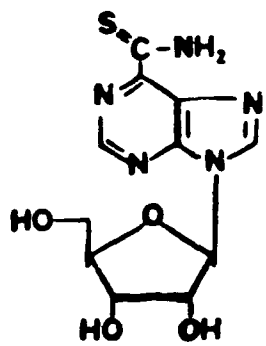
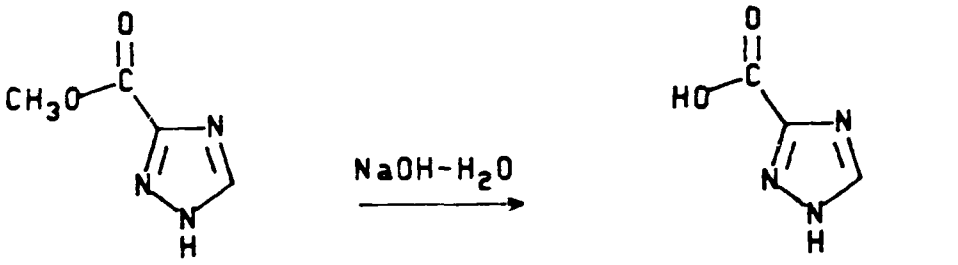


AVS 79

IV. **PROCEDURES FOR TARGET COMPOUNDS DELIVERED TO USAMRIID from January 17, 1988 to January 16, 1989**

1. 1,2,4-Triazole-3-carboxylic acid (AVS TCOOH)

AVS TCOOH was synthesized according to the following scheme:



1 2

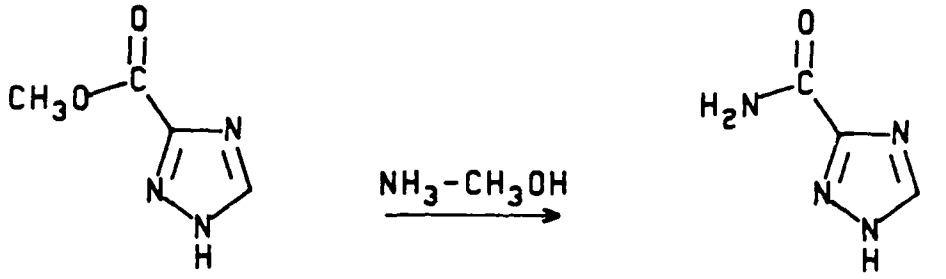
AVS-TCOOH

Experimental

1,2,4-Triazole-3-carboxylic acid: Methyl-1,2,4-triazole-3-carboxylate (6.0 g, 47 mmol) is suspended in water (50 mL) containing sodium hydroxide (3 g, 75 mmol). After stirring at room temperature for six hours the solution is adjusted to pH 4 with conc. hydrochloric acid, and the precipitated solid is collected by filtration. The crude product is recrystallized from hot water to yield 5.0 g (94%) of final product; m.p. 138-140°.

2. 1,2,4-Triazole-3-carboxamide (AVS TCA)

AVS TCA was synthesized according to the following scheme:



1 2

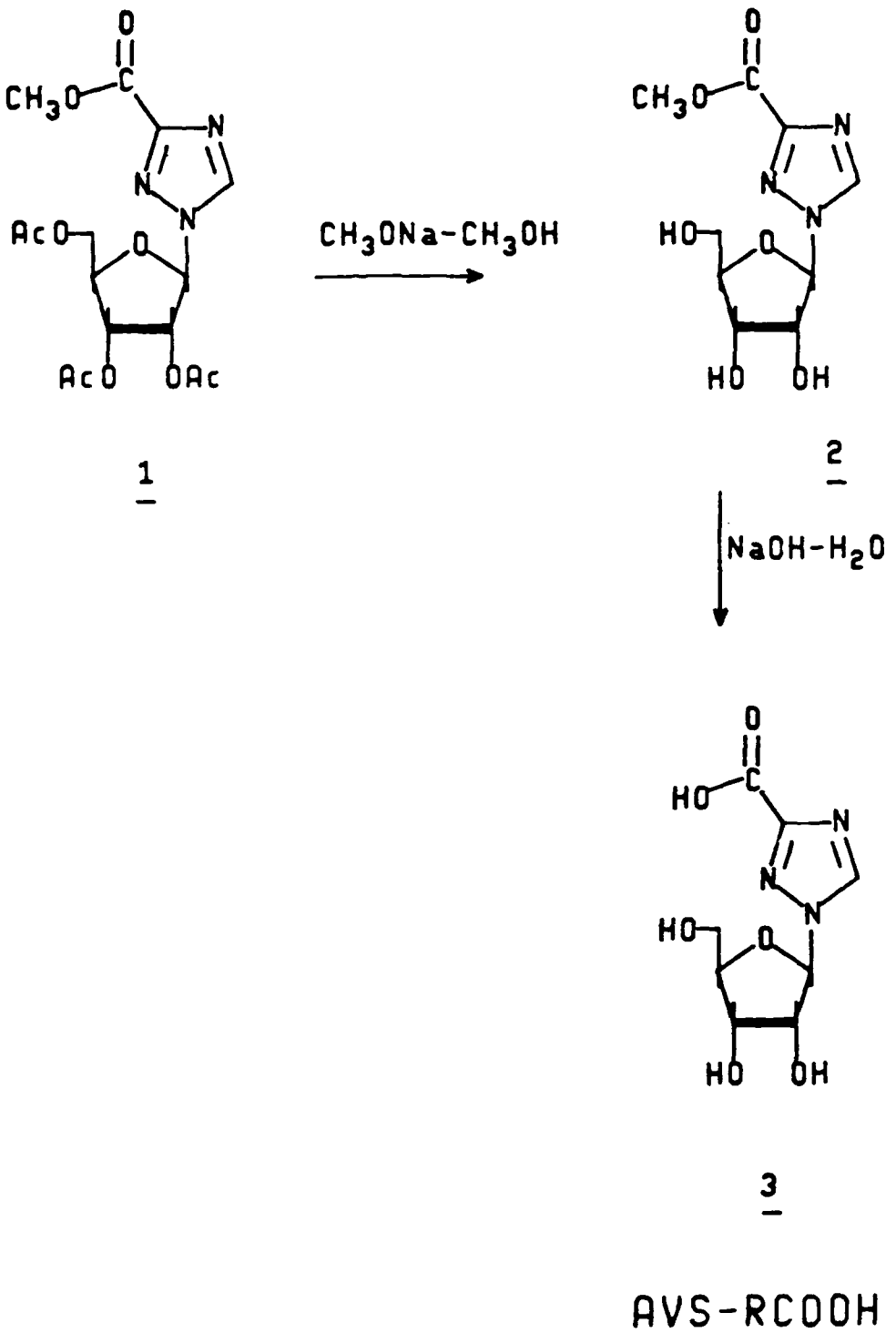
AVS-TCA

Experimental

1,2,4-Triazole-3-carboxamide: To methanol (50 mL) (saturated with ammonia at 0° in a steel bomb is added 1,2,4-triazole-3-methylcarboxylate (6.0 g, 47 mmol). After heating to 90° for 12 hours, followed by cooling to room temperature and venting, the solvent is evaporated under reduced pressure and the residue is recrystallized from hot water. Yield 4.2 g (80%); m.p. 315-317°.

3. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-methylcarboxylate

AVS-RCOOH was synthesized according to the following scheme:



1

2

3

AVS-RCOOH

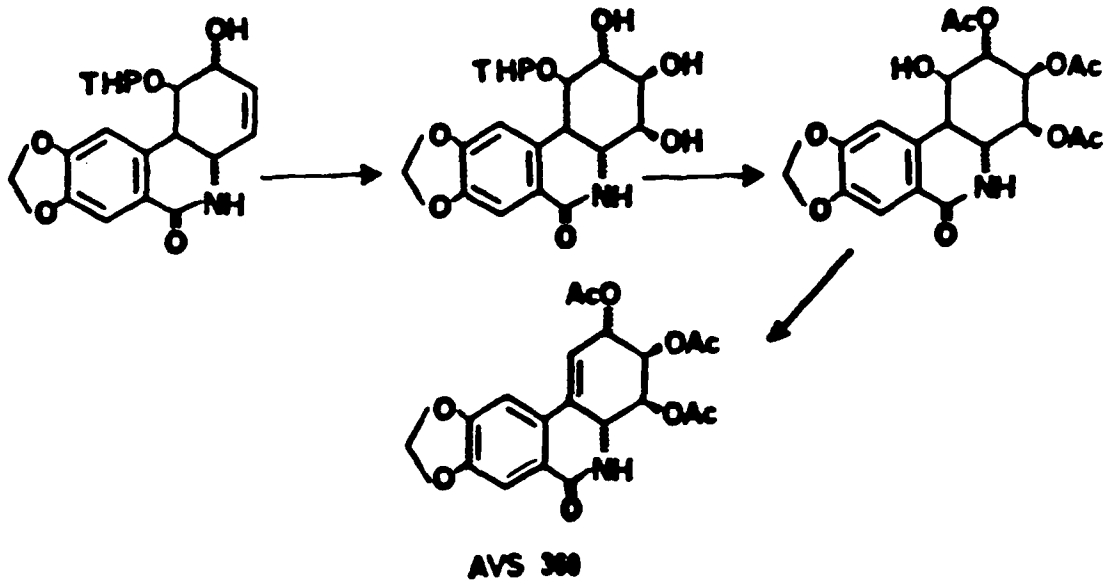
Experimental

1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-methylcarboxylate: 1-β-*D*-Tri-*O*-acetyl-ribofuranosyl-1,2,4-triazole-3-methylcarboxylate (20 g, 51 mmol) is dissolved in methanol (400 mL), then sodium methoxide (200 mg) is added to adjust the pH value of the solution to about 9.5. After stirring for one hour the solution is neutralized by adding Amberlite 120 H^+ resin, the resin is filtered off, washed with methanol, and the solvent is evaporated under reduced pressure. The residue is recrystallized from ethyl acetate (100 mL) to yield 12 g (90%) pure product, m.p. 118-120°.

1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-carboxylic acid: 1-β-*D*-Ribofuranosyl-1,2,4-triazole-3-methylcarboxylate (7.77 g, 30 mmol) is dissolved in water (20 mL), sodium hydroxide (1.4 g, 35 mmol) is added, and the solution is stirred or 1 hour. After neutralizing with Amberlite 120 H^+ resin the reaction mixture is filtered, the filtrate is evaporated to dryness under reduced pressure, and the residue is recrystallized from hot water to yield 5.0 g (68%) of final product, m.p. 196-198°.

4. Lycoricidine triacetate (AVS 360)[1]

AVS 360 was synthesized according to the following scheme:



AVS 360

Experimental

4aH-r, 1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-(2'-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (15): To a mixture of N-methylmorpholine-N-oxide (5.5 g, 47 mmol) in t-butanol (30 mL), acetone (30 mL) and water (20 mL) is added osmium tetroxide (100 mg, 0.36 mmol), followed by the addition of a solution of intermediate 14 (10.0 g, 85 mmol) in t-butanol (500 mL) and acetone (200 mL). After stirring at room temperature for 24 hours additional N-methylmorpholine-N-oxide (4.0 g, 34 mmol) and osmium tetroxide (100 mg, 0.36 mmol) is added. After 48 hours TLC indicates the completion of the reaction and the solvent is evaporated under reduced pressure. The residual solvent is coevaporated with ethanol twice (50 mL), and upon trituration with ethanol (30 mL) the crystalline product is collected by filtration. Yield: 8.1 g (74%) m.p. 218°

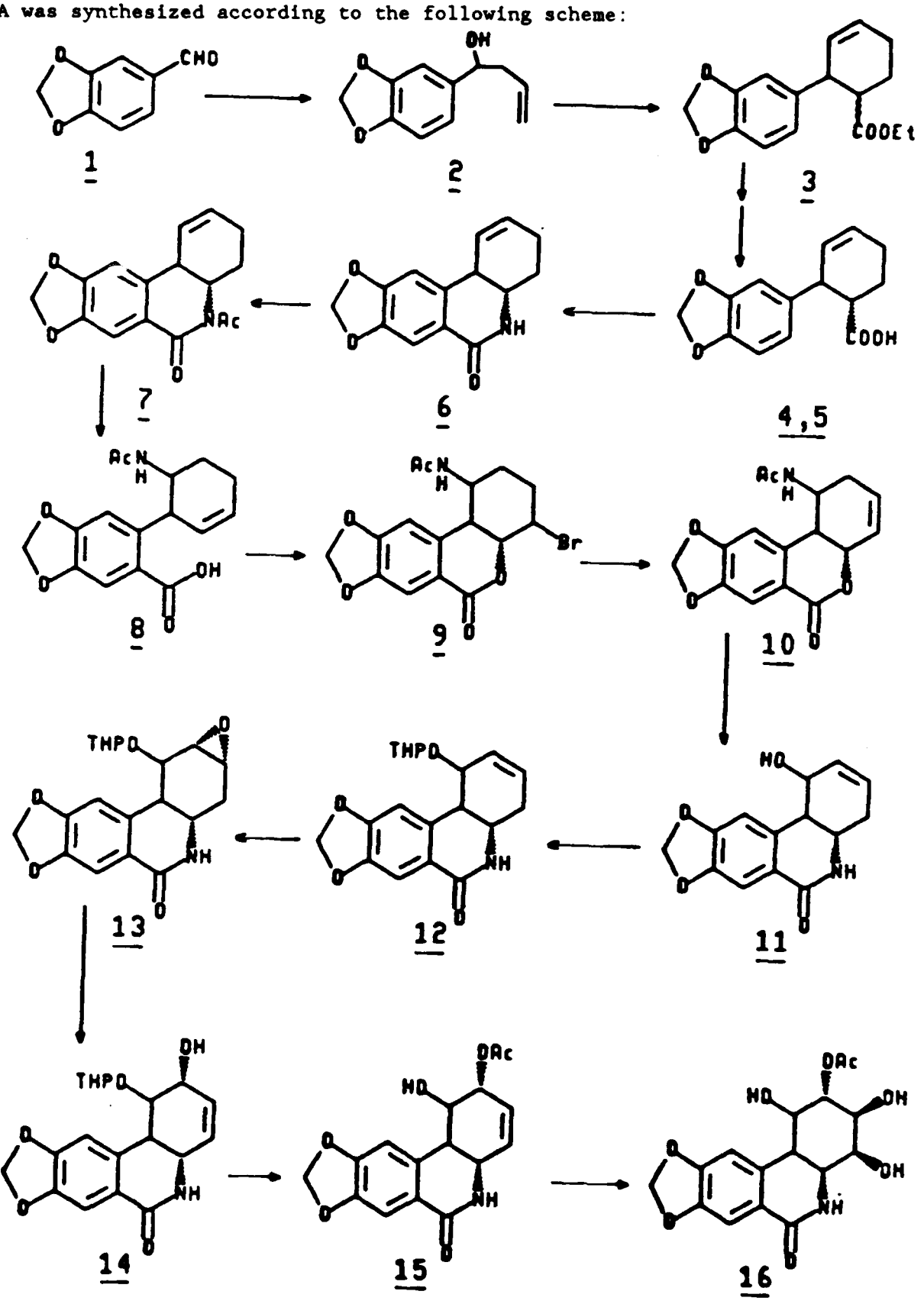
4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (16): Intermediate 15 (4.5 g, 11.4 mmol) is added to a mixture of dimethylaminopyridine (0.4 g) in acetic anhydride (200 mL). The solution is heated and kept at reflux for 2 hours, then the excess acetic anhydride is removed under reduced pressure. The residual solvent is coevaporated with ethanol (50 mL) twice, the solid product is suspended in ethanol (50 mL), cooled in an ice bath and filtered off.

The obtained product is suspended in ethanol (150 mL), together with para-toluene sulfonic acid (0.4 g). After keeping the mixture at reflux for two hours, the solution is cooled and the precipitated solid is collected by filtration. The mother liquor is concentrated and a second crop can be collected. Yield: 3.2 g (70%) m.p. 301-302°C.

Lycoricidine Triacetate (4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,-10bH-trans,2,3,4-Triacetoxy-8,9-methylenedioxy-2,3,4,4a-tetrahydro-6(5H)-phenanthridone) (17): Over a 5 minute period thionyl chloride (3.4 mL) is added to a cooled solution of intermediate 16 (2.3 g, 5.2 mmol) in pyridine (30 mL). After stirring overnight TLC indicates completion. Methylene chloride (150 mL) is added and the organic layer is washed with water (2 x 75 mL), 10% hydrochloric acid (2 x 100 mL) and water (2 x 50 mL). The organic phase is dried over sodium sulfate, the solvent is evaporated, and the residue is recrystallized from ethanol (40 mL). The crystalline material is collected by filtration and dried in air. Yield: 1.8 g (80%) m.p. 273-275°C

5. 1-Hydroxy-2-acetyl-lycoricidine (AVS 360MA)[1]

AVS-360MA was synthesized according to the following scheme:



Experimental

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofurane (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%).
(Overall yield 29%)

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4):
A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r,10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r,1H-trans,10bH-cis,4H-trans,1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r,1H-trans,10bH-cis,1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydrodibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone 9 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurred with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurred in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r,1H-trans,1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylaminolactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone 11 (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 l) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofurane (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°.

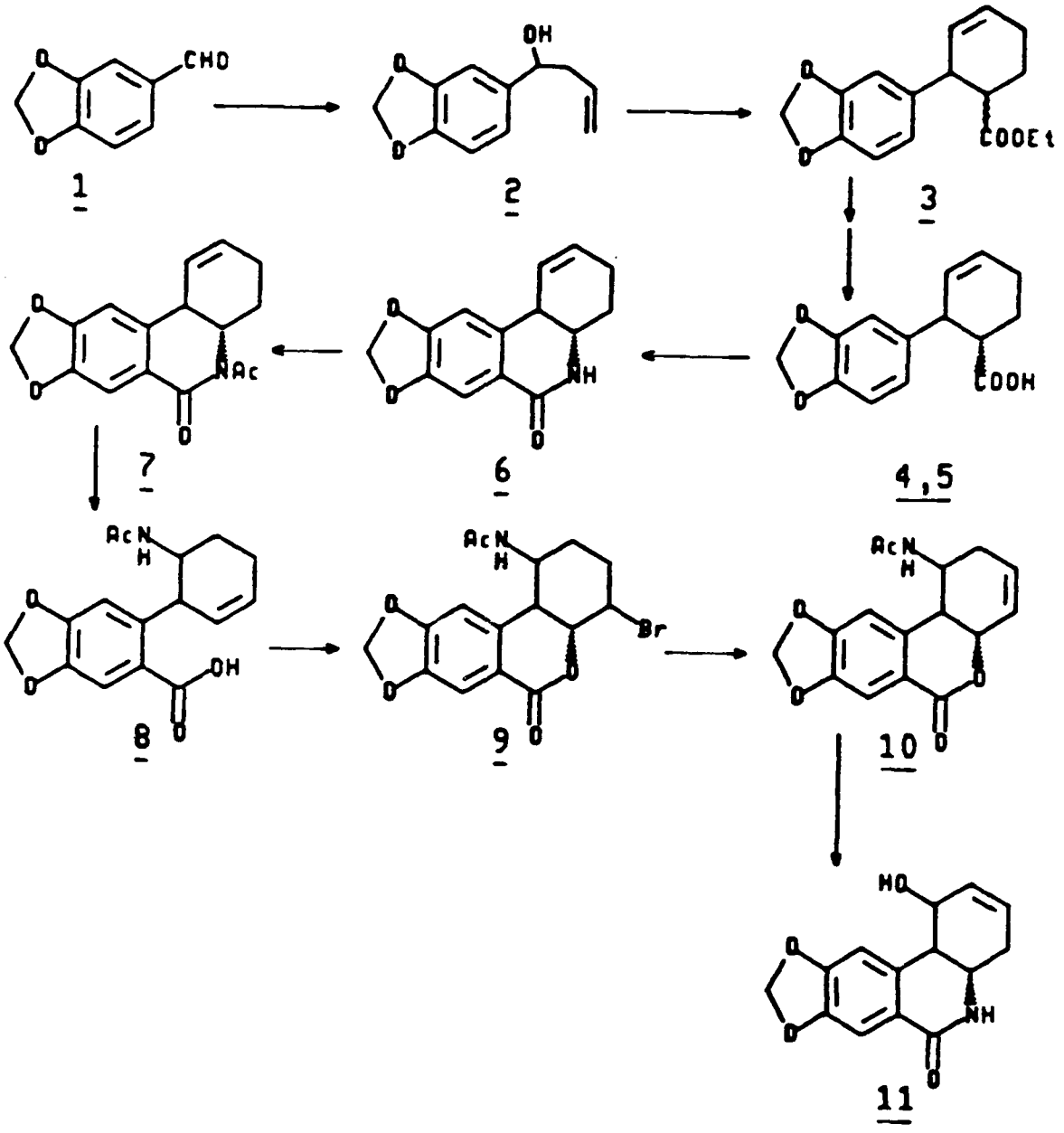
The product (7 g) is recrystallized from ethanol to give 6.7 g pure product.

4aH-r,1H-trans,2H-cis,10bH-trans,1-Hydroxy-2-acetoxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone (15): A mixture of intermediate 14 (13.0 g, 35 mmol), acetic anhydride (200 mL), and dimethylaminopyridine (300 mg) are kept at reflux for 30 minutes. The excess acetic anhydride is evaporated under reduced pressure, and the residual acetic anhydride is coevaporated with ethanol (40 mL) twice. The obtained product is suspended in ethanol (250 mL), p-toluene-sulfonic acid (500 mg) is added and the mixture is kept at reflux for 3 hours. After cooling the product is collected by filtration, and upon concentrating the filtrate a second precipitate is obtained. Yield: 9.5 g (83.3%) m.p. 291° (dec.)

4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,3,4-Trihydroxy-2-acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (16):
A solution of intermediate 15 (9.2 g, 30 mmol) in dimethylformamide (500 mL) is combined with a solutin of osmium tetroxide (100 mg) and N-methylmorpholine N-oxide (6.0 g, 55 mmol) in acetone (30 mL), t-butanol (40 mL) and water (20 mL). After 25 hours additional N-methylmorpholine N-oxide (1.5 g, 1.25 mmol) and osmium tetroxide (50 mg) are added, and the mixture is left over a three-day period. The solvent is evaporated under reduced pressure below 35°, and the residue is purified on a silica gel column with dichloromethane-methanol 9:1 (6 L) as the eluant. The combined fractions containing monoacetyl product 16 are combined, the volume is reduced to ca. 50 mL, and the resulting crystalline precipitate is collected by filtration to give 4.2 g (41.3%) of compound 16, m.p. 288-290° (decomp.).

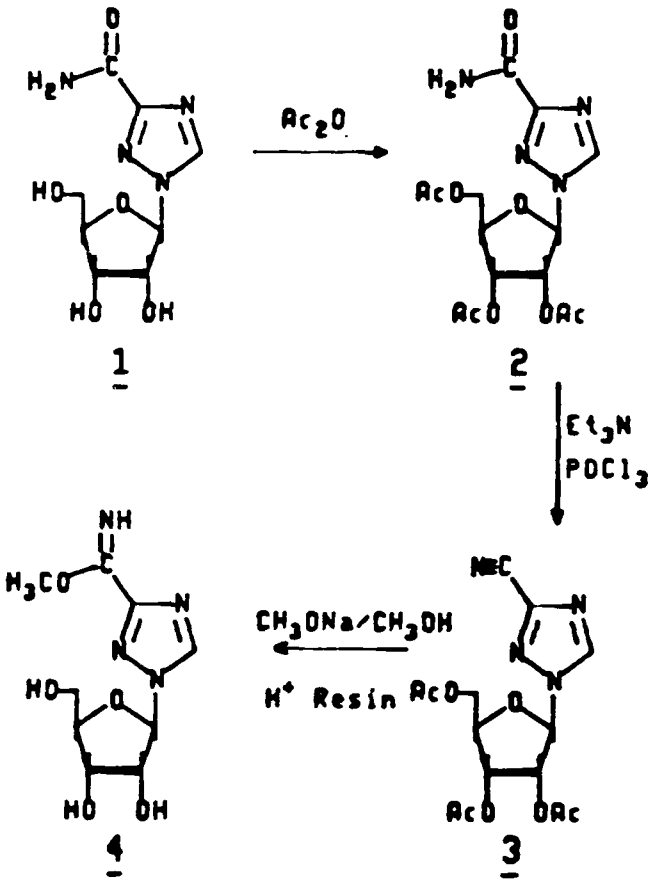
6. 1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (Intermediate II)[1]

Intermediate II was obtained as an intermediate during the synthesis of lycoricidine, according to the scheme shown.



7. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-methylamidate (AVS 4071)

AVS 4071 was synthesized according to the following scheme:



Experimental

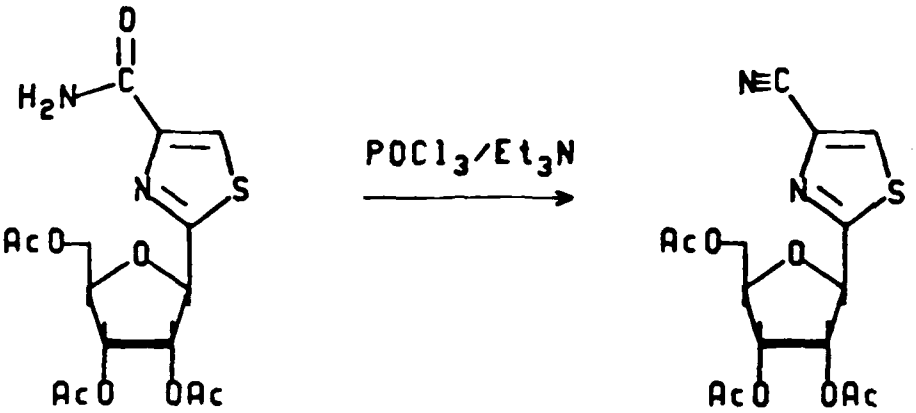
1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (3): A mixture of ribavirin (50 g, 0.2 mol), acetic anhydride (600 mL), and 4,4-dimethylaminopyridine (1 g) is stirred for 60 hours. Unreacted acetic anhydride is evaporated under reduced pressure at 40°. The obtained viscous residue is treated with ethanol (500 mL), and upon evaporation of the solvent the product is dissolved in cold water. The aqueous phase is extracted with ethyl acetate (3 x 300 mL). The combined organic layer is dried over sodium sulfate, and evaporated to give a solid foam which shows as a single spot on TLC.

Without further purification the obtained acetyl ribavirin (74 g, 0.2 mol) is combined with triethylamine (411 mL, 2.9 mol) and dissolved in chloroform (1200 mL) while cooling the solution to 0°. Phosphorous oxychloride (52 mL, 0.55 mol) is added dropwise over a period of 30 minutes. The reaction mixture is kept at 0° for another 30 minutes, then the ice bath is removed and the reaction is stirred at room temperature for 3 hours. The solvent is evaporated under reduced pressure, then the residue is dissolved in ethyl acetate (1500 mL). The ethyl acetate solution is washed with water (2 x 500 mL) and with saturated sodium bicarbonate solution. The aqueous washings are combined and extracted with ethyl acetate (200 mL), the combined organic layers are dried over sodium sulfate, and treated with charcoal at room temperature. After filtering through a Celite bed the filtrate is evaporated to dryness to yield a colored syrup. The syrup is dissolved in dichloromethane (300 mL) then the solution is passed through a short column packed with silica gel, and eluted with ethyl acetate/dichloromethane 4:1 (500 mL). After evaporation of the solvent a white, crystalline material is obtained which is homogeneous on TLC. Yield: 48.2 g (68%) m.p. 96-98°; lit. 96-97°.

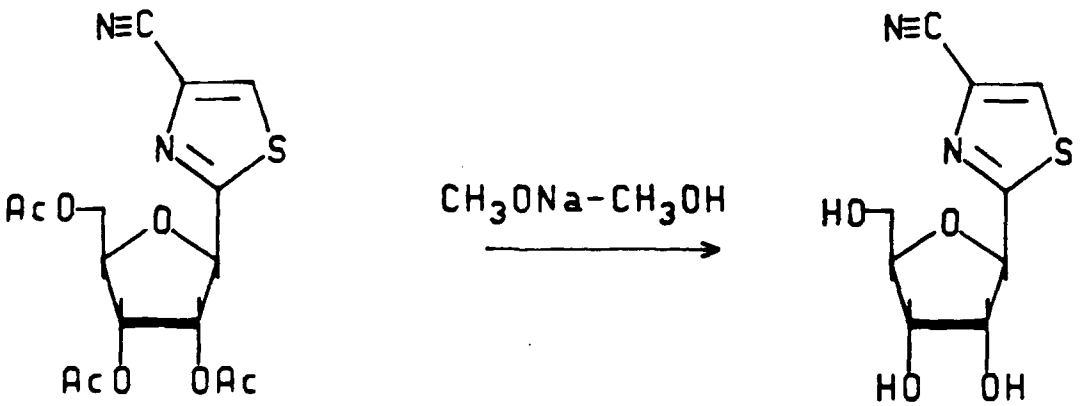
1-β-D-Ribofuranosyl-1,2,4-triazole-3-methylamidate (4): Cyano compound 3 (45 g, 0.128 mol) is dissolved in methanol (1 L), then sodium methoxide (1 g) is added to adjust the pH to 9.0. The reaction mixture is stirred for 90 minutes, then it is neutralized with ion exchange resin H^+- form. After filtration the solvent is evaporated under reduced pressure, and the residue is recrystallized from methanol to yield the pure imino ether 4; yield 22 g (66.7%), m.p. 141-142°.

8,9. Protected and Deprotected Tiazofurin nitrile (AVS ATN, AVS TFN).

AVS ATN and AVS-TFN were synthesized according to the following scheme:



AVS-TFN Ac



AVS-TFN

Experimental

2-(2,3,5-Tri-*O*-acetyl-β-*D*-ribofuranosyl)thiazole-4-carbonitrile

A solution of triacetyl-tiazofurin (19.3 g, 50 mmol) in methylene chloride (400 mL) is cooled to 0°C, triethylamine (102.7 mL) is added, followed by addition of phosphorus oxychloride (13 mL, 137 mmol). During a two hour period the solution is allowed to warm to room temperature while stirring, then the solvent is removed under reduced pressure. The oily residue is suspended in water (500 mL) and extracted with methylene chloride (2 x 250 mL). The combined methylene chloride extracts are washed with water and dried over sodium sulfate. After evaporation of the solvent the obtained syrup is passed through a silica gel column, with methylene chloride containing 5% acetone as the mobile phase. The fractions containing the desired product are combined, the solvent is evaporated and the product is obtained as a viscous syrup, as reported in the literature.

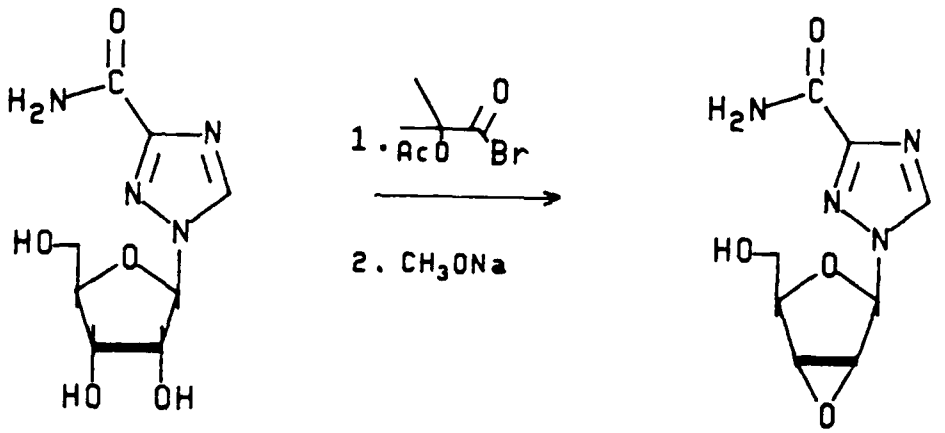
Yield 16.5 g (90.5%)

2-β-*D*-Ribofuranosyl-thiazole-4-carbonitrile

To a solution of Tri-*O*-acetyl-β-*D*-ribofuranosyl-thiazole-4-carbonitrile (50 g, 0.136 mol) in methanol (600 mL) is added sodium methoxide (200 mg) to bring the pH to 9.5. The solution is stirred for about 20 minutes while monitoring its progress by TLC. (At this stage great care has to be taken to avoid the formation of the amidic ester). After completion the solution is neutralized with H^+ resin, filtered, and evaporated to dryness. The obtained crystalline solid is recrystallized from methanol/ethyl acetate to yield 23.5 g (70%); m.p. 129-131°.

10. 1-(2',3'-Anhydro-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (AVS 4603)

AVS-4603 was synthesized according to the following scheme:[2]



1 2

Experimental

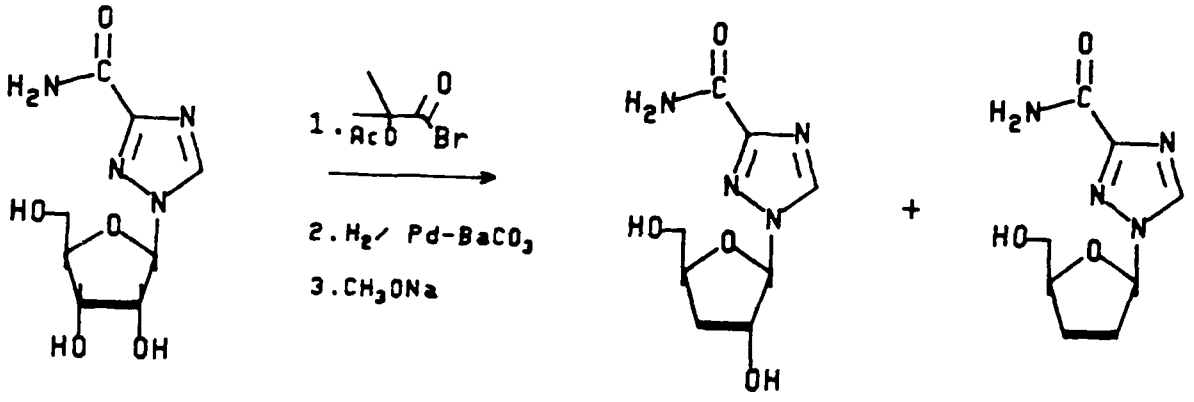
1-(2,3-Anhydro-β-D-ribofuranosyl)1,2,4-triazole-3-carboxamide

Ribavirin (2.44 g, 10 mmol) is dissolved in acetonitrile (30 mL) containing water (0.18 mL). While stirring α-acetoxyisobutyryl bromide (4.5 mL, 30 mmol) is added in one portion. After 2 hours at room temperature ethyl acetate (200 mL) is added, the solution is washed with sodium bicarbonate solution 5% (2 x 50 mL), the bicarbonate solution is extracted with ethyl acetate (100 mL) and the combined ethyl acetate extracts are washed with water (2 x 50 mL) and saturated brine solution.

The organic phase is dried over sodium sulfate, filtered, and upon evaporation of the solvent 5 g of crude material is obtained. The crude product (5 g) is dissolved in 1 M methanolic sodium methoxide solution (40 mL) and stirred for two hours, during which time a solid separated from solution. The solid is collected by filtration and recrystallized from water to yield 1.8 g (80%) of final product, m.p. 233-235°.

11. 3'-Deoxyribavirin (AVS 4602)

AVS 4602 was synthesized according to the following scheme:[2]



1 2 3

Experimental

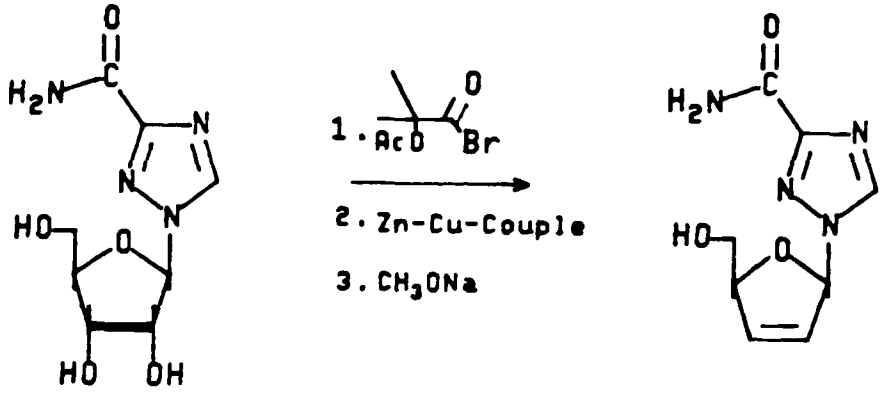
3'-Deoxyribavirin: Ribavirin (4.88 g, 20 mmol) is dissolved in acetonitrile (60 mL) and α-acetoxyisobutyryl bromide (9 mL, 50 mmol) is introduced in one portion. The reaction mixture is stirred for two hours at room temperature, then ethyl acetate (300 mL) is added to the clear solution. The organic layer is washed with 5% sodium bicarbonate solution (2 x 50 mL), the bicarbonate phase is washed with ethyl acetate (100 mL), and the combined organic phases are washed with water (2 x 50 mL) and saturated brine (50 mL). The ethyl acetate solution is dried over sodium sulfate, and the solvent is evaporated under reduced pressure to yield 9.2 g of a viscous oil.

The crude material is dissolved in dry methanol (200 mL), then triethylamine (3 mL) is added, followed by 5% Palladium on barium carbonate (2 g). The reaction mixture is hydrogenated at room temperature and atmospheric pressure for two hours, then stirring is continued for four more hours. The catalyst is filtered off, the solvent is removed under reduced pressure and the obtained residue is vacuum-dried. After dissolving the residue in methanol (200 mL) sodium methoxide (1.5 g) is added, and after two hours TLC indicates the completion of deblocking. Thin layer chromatography (methylene chloride/methanol 6:1 on silica gel) indicates the presence of two products, where the spot with the higher Rf value represents dideoxy-didehydro-ribavirin while the major spot at low Rf indicates 3'-deoxy-ribavirin, as shown by comparison with authentic samples.

The solvent is evaporated under reduced pressure, the residue is loaded onto a silica gel column and eluted with methylene chloride, gradually increasing its polarity by adding methanol. Collecting the fractions containing the two compounds, 0.5 g of dideoxy-didehydro-ribavirin and 2.1 g (47%) of 3'-deoxy-ribavirin is obtained, m.p. 141-142°.

12,13. 1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)1,2,4-triazole-3-carboxamide (AVS 4600)

AVS-4600 was synthesized according to the following scheme:[2]



1 2

Experimental

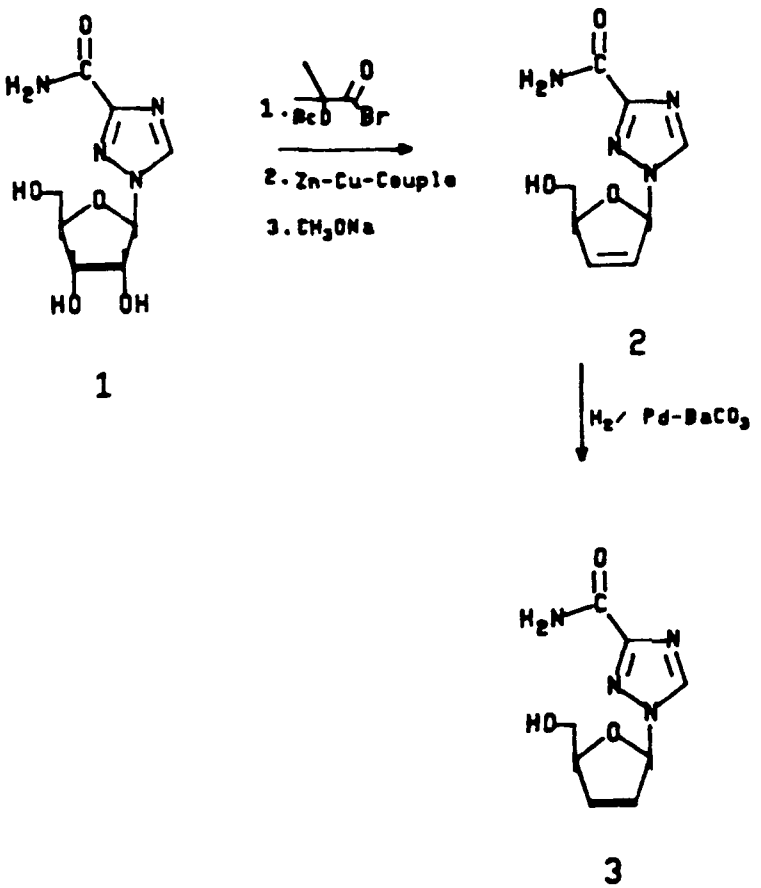
1-(2,3-Dideoxy-β-D-glycero-pent-2-eno-furanosyl)1,2,4-triazole-3-carboxamide

Ribavirin (19.5 g, 80 mmol) is dissolved in acetonitrile (200 mL) containing water (1.44 mL, 80 mmol). To this solution is added α-acetoxyisobutyryl bromide (49.4 g, 36 mL, 240 mmol) in one portion, and stirring is continued at room temperature for two hours. After adding sodium bicarbonate solution the mixture is extracted with ethyl acetate (2 x 200 mL), and the organic phase is washed with bicarbonate solution and with brine. After evaporation of the solvent under reduced pressure a highly viscous foam is obtained, which is dissolved in tetrahydrofuran (600 mL). Zinc/copper couple (80 g) is added, followed by ammonium chloride (50 g) and the reaction mixture is stirred for two hours when the temperature raises to 40°. The zinc/copper couple is filtered off, washed with ethyl acetate and the organic layer is washed with a 5% aqueous solution of ethylenediamine tetraacetic acid sodium salt, followed by washings with bicarbonate (100 mL) and brine (200 mL).

The solvent is removed under reduced pressure, the residue is dissolved in methanol (200 mL) and sodium methoxide (0.5 g) is added to adjust the pH to 9.5. After stirring for three hours a solid starts to precipitate. The solvent volume is reduced to half its volume, the precipitate is collected by filtration and recrystallized from methanol-ethyl acetate.
Yield 7.0 g (42%); m.p. 152-153°

14,15. 2',3'-Dideoxyribavirin (AVS 4601)

AVS-4601 was synthesized according to the following scheme:[2]



1 2 3

Experimental

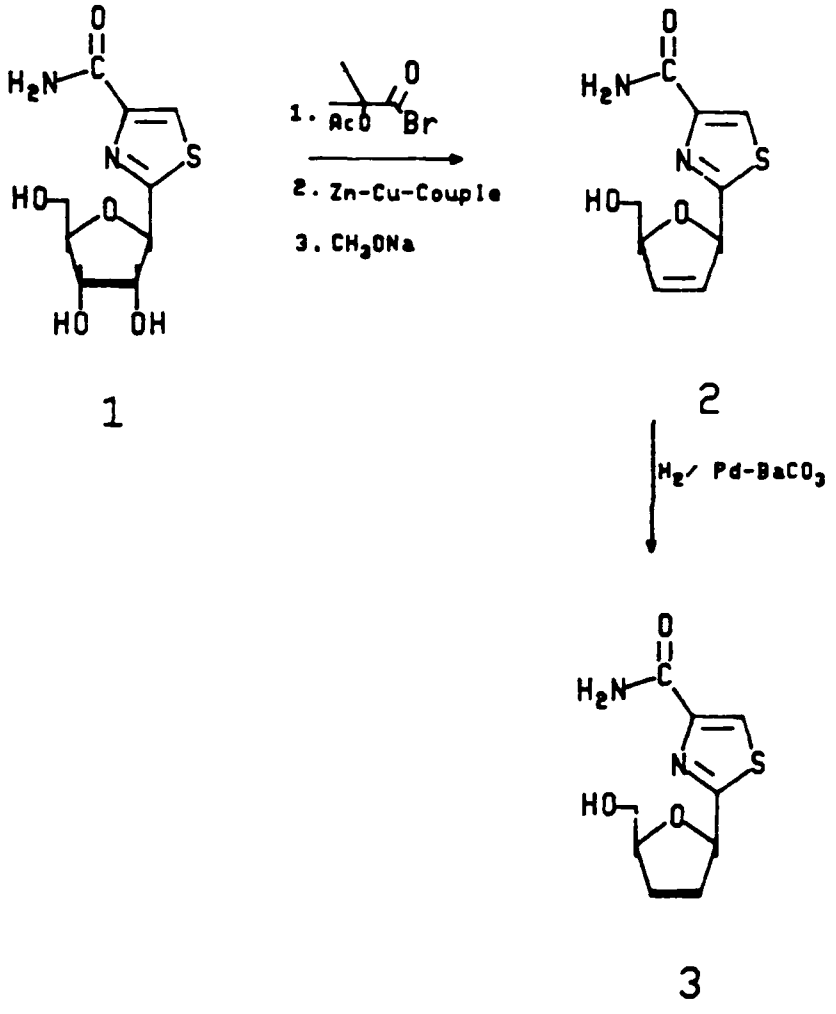
1-(2,3-Dideoxy-β-D-glycero-pent-2-eno-furanosyl)1,2,4-triazole-3-carboxamide: Ribavirin (19.5 g, 80 mmol) is dissolved in acetonitrile (200 mL) containing water (1.44 mL, 80 mmol). To this solution is added α-acetoxyisobutyryl bromide (49.4 g, 36 mL, 240 mmol) in one portion, and stirring is continued at room temperature for two hours. After adding sodium bicarbonate solution the mixture is extracted with ethyl acetate (2 x 200 mL), and the organic phase is washed with bicarbonate solution and with brine. After evaporation of the solvent under reduced pressure a highly viscous foam is obtained, which is dissolved in tetrahydrofuran (600 mL). Zinc/copper couple (80 g) is added, followed by ammonium chloride (50 g) and the reaction mixture is stirred for two hours when the temperature raises to 40°. The zinc/copper couple is filtered off, washed with ethyl acetate and the organic layer is washed with a 5% aqueous solution of ethylenediamine tetraacetic acid sodium salt, followed by washings with bicarbonate (100 mL) and brine (200 mL).

The solvent is removed under reduced pressure, the residue is dissolved in methanol (200 mL) and sodium methoxide (0.5 g) is added to adjust the pH to 9.5. After stirring for three hours a solid starts to precipitate. The solvent volume is reduced to half its volume, the precipitate is collected by filtration and recrystallized from methanol-ethyl acetate.
Yield 7.0 g (42%); m.p. 152-153°

2'-3'-Dideoxyribavirin: Ribavirin-2'-ene (2.3 g, 11 mmol) is dissolved in methanol (100 mL), and palladium on barium carbonate (500 mg) is added. The mixture is hydrogenated at room temperature and atmospheric pressure for 3 hours. The catalyst is filtered off on a glass-sinter funnel, the filtrate is evaporated to dryness under reduced pressure, and the residue is recrystallized from methanol (50 mL) to yield 1.8 g (77%) of dideoxyribavirin, m.p. 153-154°.

16. 2',3'-Dideoxytiazofurin (AVS 4604)

AVS 4604 was synthesized according to the following scheme:[2]



1 2 3

Experimental

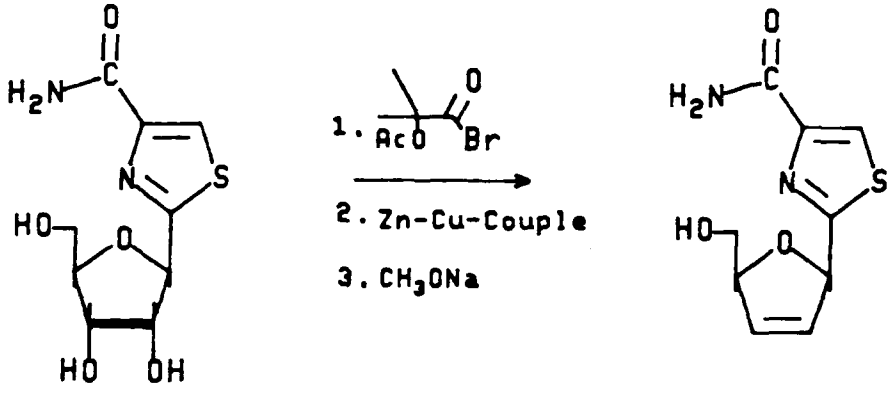
2',3'-Dideoxy-2',3'-didehydro-tiazofurin: Tiazofurin (5.12 g, 20 mmol) is suspended in acetonitrile (60 mL) containing water (0.36 mL), and α-acetoxy-isobutyryl bromide (9 mL, 60 mmol) is added in one portion. After stirring at room temperature for three hours ethyl acetate (200 mL) is added, and the solution is washed with 5% sodium bicarbonate (2 x 50 mL). The aqueous layer is extracted with ethyl acetate (100 mL), the combined organic layers are washed with water (2 x 50 mL) and brine (50 mL), followed by drying over sodium sulfate.

The solvent is evaporated under reduced pressure and the obtained solid foam is dissolved in tetrahydrofuran (200 mL). Zinc-copper couple (25 g) and ammonium chloride (12 g) are added and the mixture initially at 40°, is stirred while allowing the temperature to adjust to room temperature. After 2½ hours the Zn/Cu-couple is filtered off, the solvent is evaporated under reduced pressure, and the residue is taken up in ethyl acetate (300 mL). The solution is washed with 5% EDTA solution (2 x 50 mL), the aqueous phase is extracted with ethyl acetate (100 mL) and the combined organic layers are washed with water (100 mL) and brine (50 mL). After drying over sodium sulfate the solvent is evaporated under reduced pressure, the residue is dissolved in methanol (100 mL), and sodium methoxide (0.5 g)is added to adjust the pH to 10. After stirring for two hours TLC indicates complete disappearance of starting material and Amberlite H^+ resin is added to neutralize the medium. The resin is filtered off, the solvent is evaporated under diminished pressure, and the crude residue is loaded on a silica gel column. The product is eluted with dichloromethane containing 5% methanol, and the combined fractions containing the dideoxy product are evaporated. Recrystallization from ethyl acetate gives 3.9 g (86%) of pure product, m.p. 120-121°.

2',3'-Dideoxy-tiazofurin: Tiazofurin-2'-ene (2.5 g, 10 mmol) is dissolved in methanol (100 mL), kept under nitrogen. Carefully 5% palladium on barium carbonate (1 g) is introduced, and the hydrogenation is carried out at room temperature and atmospheric pressure during a two hour period. The catalyst is filtered off, the solvent is evaporated under reduced pressure and the residue is recrystallized from ethyl acetate; yield 2.1 g (84%); m.p. 94-95°. Analysis shows that the compound crystallizes with 0.5 mol of water.

17. 2',3'-Dideoxy-2',3'-didehydrotiazofurin (AVS 4606)

AVS 4606 was synthesized according to the following scheme:[2]



1 2

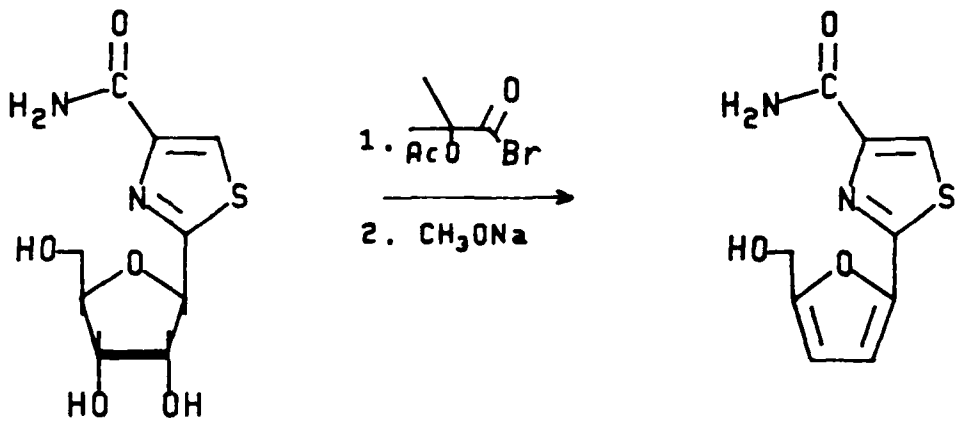
Experimental

2-(2',3'-Dideoxy-β-D-glycero-pent-2-eno-furanosyl)-thiazole-4-carboxamide: Thiazofurin (5.12 g, 20 mmol) is suspended in acetonitrile (60 mL) containing water (0.36 mL), and α-acetoxyisobutyryl bromide (9 mL, 60 mmol) is added in one portion. After stirring at room temperature for three hours ethyl acetate (200 mL) is added, and the solution is washed with 5% sodium bicarbonate (2 x 50 mL). The aqueous layer is extracted with ethyl acetate (100 mL), the combined organic layers are washed with water (2 x 50 mL) and brine (50 mL), followed by drying over sodium sulfate.

The solvent is evaporated under reduced pressure and the obtained solid foam is dissolved in tetrahydrofuran (200 mL). Zinc-copper couple (25 g) and ammonium chloride (12 g) are added and the mixture initially at 40°, is stirred while allowing the temperature to adjust to room temperature. After 2½ hours the Zn/Cu-couple is filtered off, the solvent is evaporated under reduced pressure, and the residue is taken up in ethyl acetate (300 mL). The solution is washed with 5% EDTA solution (2 x 50 mL), the aqueous phase is extracted with ethyl acetate (100 mL) and the combined organic layers are washed with water (100 mL) and brine (50 mL). After drying over sodium sulfate the solvent is evaporated under reduced pressure, the residue is dissolved in methanol (100 mL), and sodium methoxide (0.5 g)is added to adjust the pH to 10. After stirring for two hours TLC indicates complete disappearance of starting material and Amberlite H^+ resin is added to neutralize the medium. The resin is filtered off, the solvent is evaporated under diminished pressure, and the crude residue is loaded on a silica gel column. The product is eluted with dichloromethane containing 5% methanol, and the combined fractions containing the dideoxy product are evaporated. Recrystallization from ethyl acetate gives 3.9 g (86%) of pure product, m.p. 120-121°.

18. 2-(5'-Hydroxymethylfuran-2-yl)thiazole-4-carboxamide (AVS 4605)

AVS 4605 was synthesized according to the following scheme:[2]



1 2

Experimental

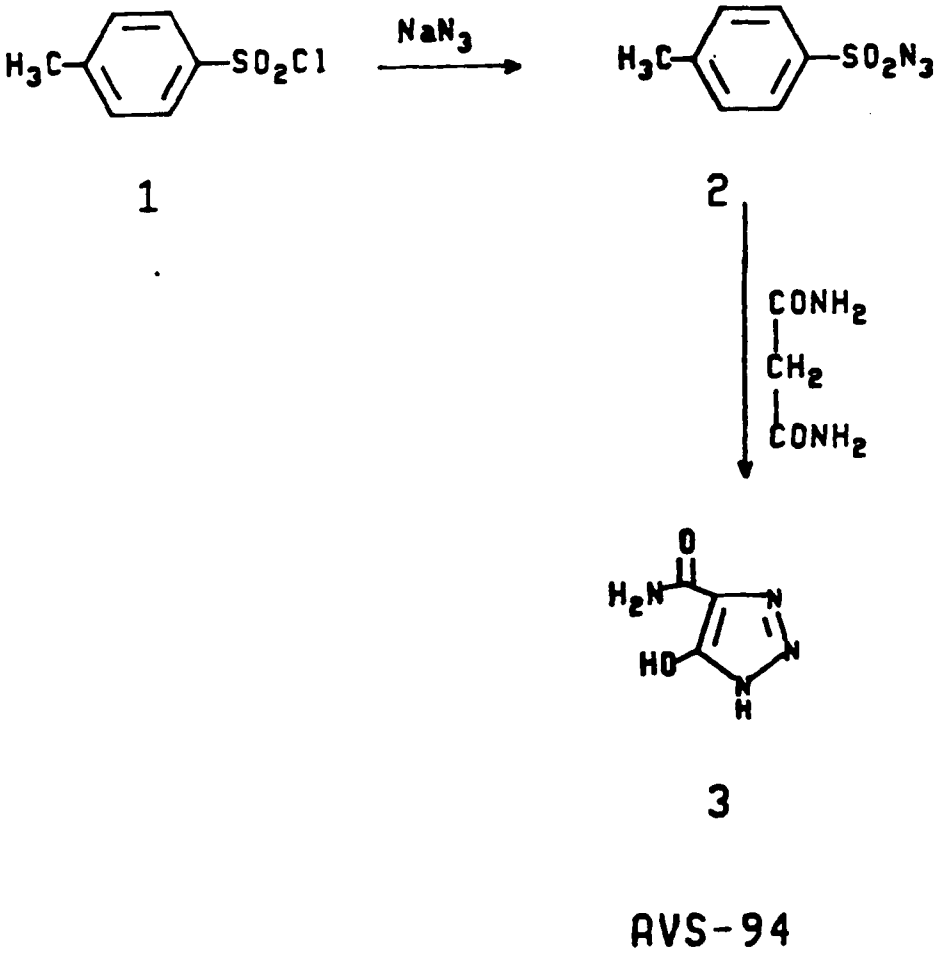
2-(5-Hydroxymethylfuran-2'-yl)thiazole-4-carboxamide: Tiazofurin (2.6 g, 10 mmol) is suspended in acetonitrile (30 mL) containing water (0.18 mL, 10 mmol) and α-acetoxyisobutyryl bromide (4.5 mL, 30 mmol) is added in one portion. The reaction mixture is stirred for two hours when a clear solution forms. Ethyl acetate (200 mL) is added, and the solution is washed with 5% sodium bicarbonate solution (2 x 50 mL), the aqueous phase is extracted with ethyl acetate (100 mL), and the combined organic extracts are washed with water (50 mL) and brine (50 mL). After drying over sodium sulfate the solvent is evaporated to yield a crude reaction product.

The crude product is dissolved in anhydrous methanol (100 mL) and sodium methoxide (1.5 g) is added to result in a pH value of 10 of the solution. After stirring for two hours at room temperature the reaction mixture is neutralized with Amberlite resin H^+ (20 g). The resin is collected by filtration, the solvent is evaporated and the residue is recrystallized from methanol (25 mL) to yield 1.9 g (85%) of pure product, m.p. 192-194°; (lit. 192-193°).

Compounds 9 through 15 were submitted for testing, and the syntheses as well as their antiviral activities will be reported at the American Chemical Society National Meeting, Los Angeles, CA, September 1988.

19. 4-Hydroxy-1,2,3-triazole-5-carboxamide (AVS-94)

AVS-94 was synthesized according to the following scheme:[3]



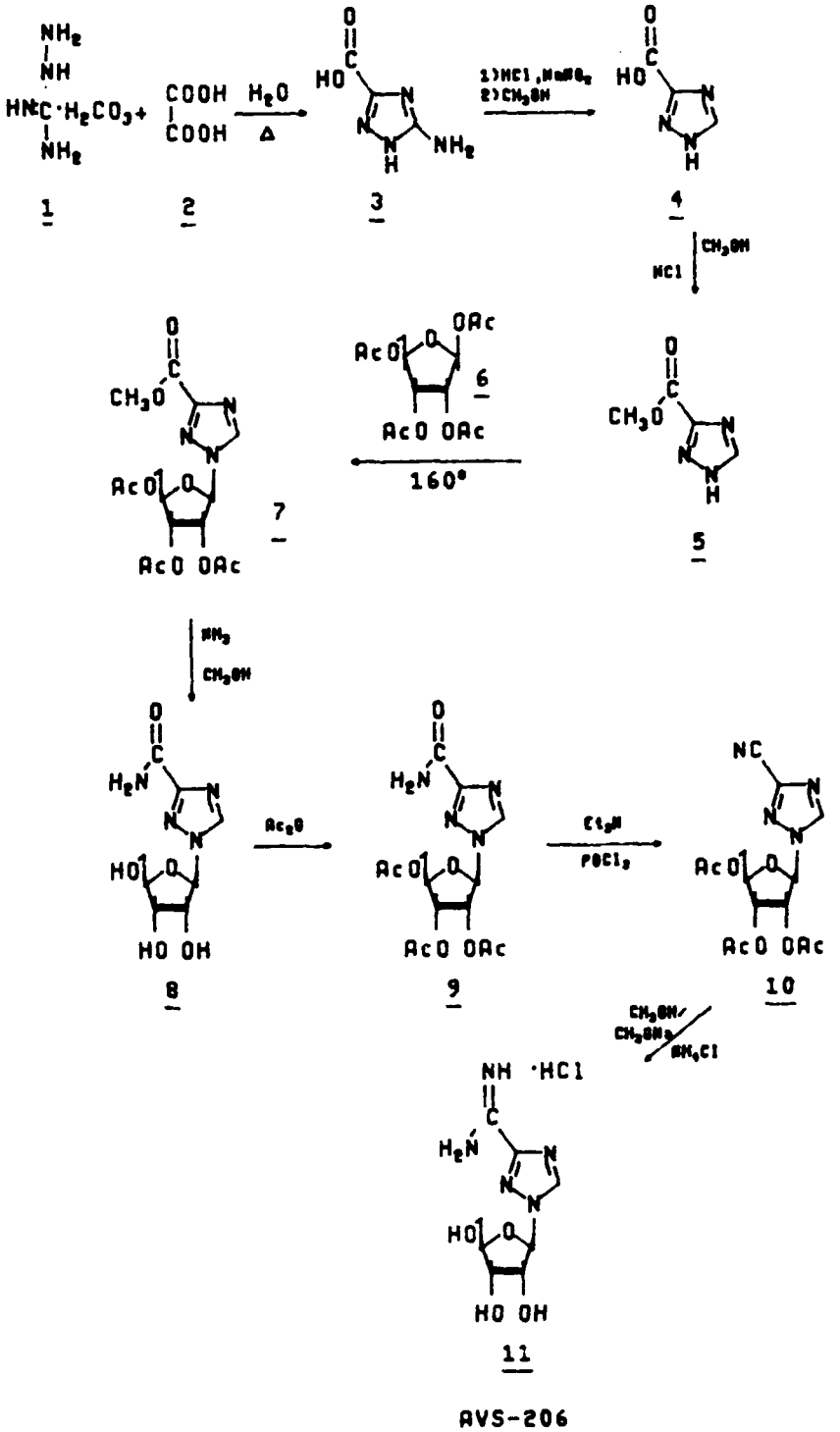
Experimental: Sodium azide (71.5 g, 1.10 mol) is dissolved in water (240 mL) and combined with ethanol (360 mL). While stirring a warm solution (45°) of p-toluene sulfonyl chloride (190 g, 1 mol) in alcohol (1 L) is added, and stirring is continued for 2½ hours. The solvent is removed under reduced pressure at 35°, the residue is taken up in water (1.2 L) and transferred to a separatory funnel. p-Toluene sulfonyl azide separates as an oil which is collected and dissolved in dichloromethane (500 mL). The organic phase is washed with brine, dried over sodium sulfate, and upon evaporation of the solvent under reduced pressure 190 g of p-toluene sulfonyl azide is obtained as an oil.

AVS 94

In a dry 3 L three-neck flask, sodium (23 g, 1 mol) is dissolved in absolute ethanol (1.5 L) and, while cooling externally malonamide (102 g, 1 mol) is added. p-Toluene sulfonyl azide (190 g, 0.99 mol) is dissolved in ethanol (200 mL) and the solution is added dropwise to the malonamide solution while stirring. After 30 minutes the reaction mixture is heated and kept at reflux for 30 minutes, then it is left to cool to room temperature. The precipitated salt is collected by filtration, the filter cake is washed with ethanol (100 mL) and ether (10 mL), and the air-dried salt is dissolved in a minimum amount of hot water (1000 mL). The pH is adjusted to pH 2 with dilute hydrochloric acid, and upon cooling the product crystallizes from solution. The crystals are filtered off, and recrystallized from hot water to give 37 g (29%) of pure compound, m.p. 190-192°, lit. 190-193°.

20. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (AVS 206)

AVS-206 was synthesized according to the following scheme:[4]



Experimental

1) 5-Amino-1,2,4-triazole-3-carboxylic acid[1] (3):

Oxalic acid (11.2 Kg, 123.6 mol) was dissolved in water (255 L). While stirring aminoguanidine bicarbonate (10.5 Kg, 77.14 mol) is added portionwise. The reaction mixture is heated to 85°, and kept at 85-92° for eight hours. Upon cooling to about 70° a solution of sodium hydroxide (13.25 Kg, 50% in 10 L water) is added, then the reaction batch is reheated and kept at reflux for 90 minutes. After cooling overnight the turbid solution is filtered. The filtrate is neutralized with hydrochloric acid (4.5 L) and the resulting precipitate is collected by filtration. The product is dried at 55° while under aspirator vacuum (3 days). The product is used in the next step without further purification.

Yield: 8.81 Kg (89%); m.p. 243° (lit. 242-244°)

2) 1,2,4-Triazole-3-carboxylic acid[2] (4):

5-Amino-1,2,4-triazole-3-carboxylic acid (1.4 Kg, 10.92 mol) is dissolved in hot hydrochloric acid (3.4 L conc. HCl and 8.4 L water). After cooling to 5°C a sodium nitrite solution (1.160 Kg sodium nitrite in 2.6 L water) is slowly added while maintaining the temperature below 10° by cooling in an ice bath. The precipitated diazo salt is collected by filtration and the filter cake is pressed dry without letting it go to complete dryness.

Caution: the dry diazocompound is explosive and it detonates violently when submitted to heat or friction.

A small amount of the moist diazonium salt is added to methanol (6 L at 35°), and upon initiation of the decomposition reaction strong cooling in an ice bath was required while maintaining the reaction in balance by adding small amounts of the diazonium salt. Upon completion the precipitated deamination product is collected by filtration and dried. Yield: 394 g m.p. 125-126°. This product is pure enough to be used in the subsequent esterification step.

3) Methyl-1,2,4-triazole-3-carboxylate[2] (5):

To a suspension of 1,2,4-triazole-3-carboxylic acid (2.125 Kg, 18.77 mol) in methanol (12 L) hydrochloric acid gas is injected while maintaining the temperature below 20° with cooling. After gas saturation the obtained solution is left at room temperature for five days. After that time the precipitated hydrochloride salt is collected by filtration, and dried, then added to water (4 L) for hydrolysis. The methyl ester is filtered and dried. The crude material is recrystallized from boiling water (8 L) to give purified crystalline methyl-1,2,4-triazole-3-carboxylate.

Total Yield: 1247 g m.p. 198-199° (lit. 198°)

4) Methyl-1-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate[3] (7):

Methyl-1,2,4-triazole-3-carboxylate (127 g, 1 mol) and 1,2,3,5-tetra-O-acetyl-ß-D-ribose (318 g, 1 mol) are mixed thoroughly and placed in a three-neck

flask equipped with a mechanical stirrer, thermometer, and take-off condenser. The flask is immersed in an oil bath preheated to 165°. After the sugar derivative has melted bis-p-nitrophenylphosphate (2.5 g) is added. After stirring for 5 minutes the pressure in the reaction apparatus is reduced and the generated acetic acid distills off. After 30 minutes the oil bath is removed, and the reaction mixture is left to cool to 50-60°.

The highly viscous, dark reaction mass is slowly poured into cold methanol (1.2 L) while stirring, and the product starts to crystallize.

Seven such fusion reactions are performed, and the combined batches are washed with methanol to give a total of 2.2 Kg (yield 61%) of methyl-1-(2,3,5-tri-O-acetyl-ß-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate.
m.p. 104-105° (lit. 107-109°). Thin layer chromatography indicates that the product is almost exclusively the ß-isomer.

5) 1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin)[3] (8):

Methanol (22 L) is placed in a stainless steel reactor and cooled to -10° by circulating alcohol at -40° through the reactor jacket. Liquid ammonia (4 L) is added to the cold methanol and the powdered acetylnucleoside 7 (2.24 Kg) is added in small portions. Upon completion of the addition the vessel is sealed air-tight, and the content is allowed to warm up to room temperature. After two days the vessel is slowly vented and the contents is heated to 35-40°, then filtered through a filter pad. The filtrate is concentrated under reduced pressure, and the precipitated solid is collected by filtration to yield 1130 g of crude ribavirin.

From the first and second mother liquor more product is obtained. Upon recrystallization from hot methanol-water 3:1 (13 L) a total yield of 1283 g (88%) is obtained. m.p. 166-167° (lit. 166-168°).

Elemental analysis, thin layer chromatography, and spectral characteristics confirm that the obtained product is the pure ß-isomer of 1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin).

6) 1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (10)[4]: A mixture of ribavirin (700 g, 2.8 mol), acetic anhydride (6 L), and 4,4-dimethylaminopyridine (14 g) is stirred for 60 hours. Unreacted acetic anhydride is evaporated under reduced pressure at 40°. The obtained viscous residue is treated with ethanol (500 mL), and upon evaporation of the solvent the product is dissolved in cold water (5 L). The aqueous phase is extracted with dichloromethane (3 x 2 L) and after drying, more

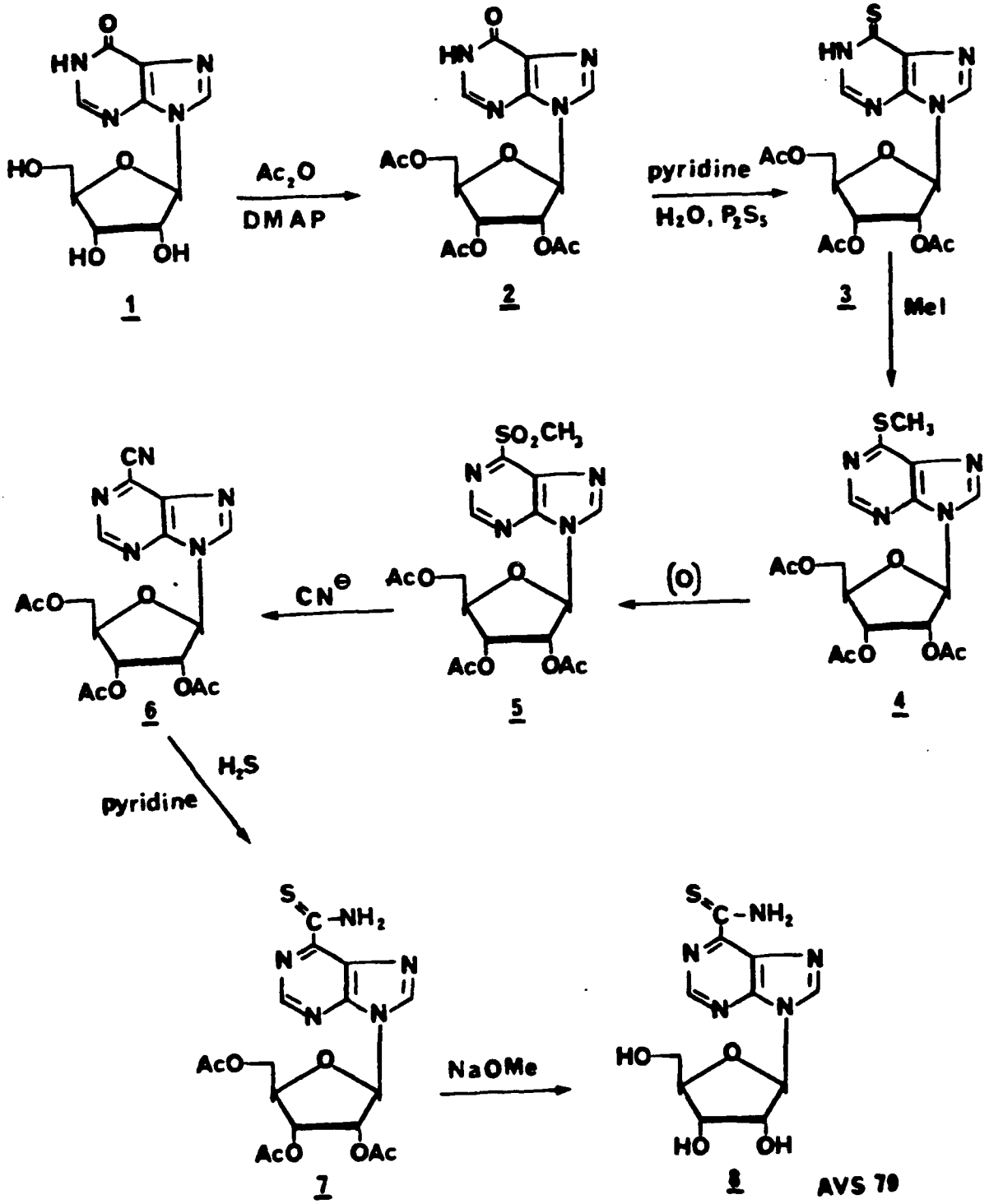
dichloromethane (4 L) and triethylamine (5.7 L, 20.6 mol) are added to the tri-O-acetylribavirin solution. After cooling the solution to 5° phosphorous oxychloride (730 mL, 7.72 mol) is added dropwise over a 50 minute period, maintaining the temperature below 8°. The mixture is allowed to warm to room temperature (1 hour) with additional stirring for 2½ hours. Ice water (5 L) and additional dichloromethane (5 L) are added, the layers are separated, and the organic layer is washed with water (2 L), dilute acetic acid (200 mL glacial acetic acid in 1800 mL water), and bicarbonate solution (100 g in 2 L water) to obtain pH 6 of the aqueous phase. The organic layer is dried, evaporated and the syrupy residue is dissolved in warm methanol (700 mL). The crystals

that separate are collected by filtration to yield 500 g of the nitrile compound. The mother liquor is subjected to column chromatography on silica gel, first with dichloromethane, then with dichloromethane/acetone 4:1 as the eluant. After using the solvent mixture (25 L) another crop (130 g) is obtained upon evaporation of the solvent. Yield 624 g m.p. 95-97°. Lit 96-97°.

7) 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (11)[4]: In a 22 L round bottom flask cyanotriazole derivative 10 (704 gr., 2 mol) is suspended in methanol (12 L). Sodium methoxide (45 g) is slowly introduced and the reaction mixture is stirred for two hours. When thin layer chromatography (Silica gel/SSE) indicates complete deprotection as well as imidate formation the sodium ions in solution are neutralized by adding H + ion exchange resin while stirring. The mixture is filtered, anhydrous ammonium chloride (107 g, 2 mol) is added to the filtrate and the reaction is kept at gentle reflux for two hours while stirring. Upon completion of the amidine formation (TLC) charcoal (15 g) is added. The mixture is stirred for 15 minutes then filtered through a Celite pad. The solution is concentrated to one third its volume and left overnight when the product crystallizes. The crystals are collected by filtration, and the mother liquor is concentrated further to yield a second crop of crystals. The two batches are combined and recrystallized from methanol to give 480 g (85.8%) of pure amidine hydrochloride. m.p. 180-181°C (lit: 179-181°).

21. 9-β-D-Ribofuranosylpurine-6-thio-carboxamide (AVS-79)

AVS-79 was synthesized according to the following scheme:[5]



Experimental

The overall procedure for the synthesis of AVS 79 was analogous to literature preparations:

1. A. Yamane, A. Matsuda and T. Ueda, Chem. Pharm. Bull., **1980**, 28, 150.

2. J. D. Westover, G.R. Revankar, R.K. Robins, R.D. Madsen, J.R. Ogden, J.A.-North, R.W. Mancuso, R.J. Rousseau, and E.L. Stephen, J. Med. Chem., 1981, 24, 94.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)hypoxanthine (Tri-O-acetylinosine) 2: A mixture of inosine (65.0 g, 0.242 mol), acetic anhydride (750 ml) and 4-N,N--dimethylaminopyridine (1.0 g) was stirred under anhydrous conditions at room temperature for 24 hours. The reaction mixture was heated in a water bath (bath temp. 75°) for 15 minutes. Unreacted acetic anhydride was evaporated under reduced pressure (bath temperature 40°C). The residue was carefully treated with methanol (300 ml) and evaporated to dryness. The white residue was triturated with ethanol (600 ml) and filtered. The white solid was washed with ethanol (2 x 100 ml), and air dried to give 94.7 g (99.7%) of 2. m.p. 244--245° (Literature m.p. 244°). All the spectral data matched reported values.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)-6-mercaptopurine (Tri-O-acetylthioinosine) 3: To a well stirred mixture of 2 (93.0 g, 0.236 mol) and pyridine (3570 ml), phosphorus pentasulfide (220.0 g, 0.49 mol) was added in one portion. Water (35.5 ml, 1.97 mol) was added slowly over a period of 15 minutes and the reaction mixture was refluxed for 7 hours. The reaction mixture was cooled to room temperature and a clear liquid was decanted from the thick viscous residue. This residue was slowly poured into boiling water (500 ml) while stirring. The previously decanted top layer was evaporated under reduced pressure to a thin syrup and added slowly to the boiling aqueous solution while stirring. After boiling for 30 minutes the reaction mixture was cooled and the solid which separated was filtered, washed with ice water (3 x 300 ml) and dried in air to yield 75.5 g (77.75%) of 3. m.p. 235-236°(dec). The structural identity of 3 was confirmed by spectral analyses.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)-6-methylthiopurine 4: To a well--stirred mixture of 3 (73.0 g, 0.178 mol) and dimethylformamide (350 ml) in a three-necked flask fitted with a mechanical stirrer and a dropping funnel was added anhydrous potassium carbonate (28.0 g, 0.202 mol). Methyliodide (57.0 g, 0.402 mol) was added slowly through the dropping funnel over 25 minutes. After stirring for 4 hours at room temperature, the reaction mixture was poured into ice-water (1000 ml) and extracted with ethyl acetate (3 x 400 ml). The organic layer was washed with saturated sodium chloride solution (2 x 400 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The syrupy residue was co-evaporated with ethyl acetate (2 x 200 ml) and the residue was kept under vacuum to form a foam which was homogeneous by TLC. Yield of 4: 63.1 g (83.5%). Spectral data of 4 agree with structural assignments.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)-6-methylsulfonylpurine 5: Method A: A solution of 4 (60.37 g, 0.142 mol) in glacial acetic acid (1800 ml) was

maintained at about 0°C while potassium permanganate (44.0 g, 0.278 mol) was added slowly over 1 hr. The flask was removed from the cooling bath and the reaction mixture was stirred at room temperature for 4 hours. The dark colored reaction mixture was poured into water (3000 ml), and saturated with sodium chloride. After extraction with ethyl acetate (5 x 500 ml), the organic layer was washed with water (3 x 1000 ml), aqueous sodium bicarbonate solution (10%) (2 x 1000 ml), and dried over sodium sulfate. Evaporation of the solvent gave 36.46 g (67.5%) of 5 as a white foam. The product was homogeneous by TLC and its spectral data agreed with its structure. The product was used for further reaction without purification. Since Method A involved laborious workup procedures and gave poor yields, the reaction was modified by replacing potassium permanganate with m-chloroperbenzoic acid as the oxidizing agent.

Method B: To a solution of 4 (24.0 g, 0.052 mol) in dichloromethane (625 ml) m-chloroperoxybenzoic acid (20.0 g, 0.11 mol) was added in one portion and the reaction mixture was stirred at room temperature for 2 hours. TLC indicated the completion of the reaction. The reaction mixture was diluted with dichloromethane (300 ml) and washed carefully with saturated sodium bicarbonate solution to remove m-chlorobenzoic acid and unreacted m-chloroperoxybenzoic acid. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give 5 as a white foam. Yield 24.0 g (93%). This product was identical to the one obtained by method A.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)-purine-6-carbonitrile 6: Finely powdered potassium cyanide (8.5 g, 0.130 mol) was added to a well-stirred solution of 5 (35.8 g, 0.0785 mol) in dimethylformamide (205 ml), and stirring was continued at room temperature for 3.5 hours under anhydrous conditions. TLC indicated the presence of some unreacted starting material. An additional 2.0 g of powdered potassium cyanide was added to the reaction mixture and stirring was continued overnight. The dark colored solution was poured into water (1000 ml), neutralized with acetic acid (4 ml), extracted with ethyl-acetate (4 x 300 ml) and the organic layer was washed with saturated sodium chloride solution (3 x 2000 ml). The organic layer was dried over sodium sulfate and evaporated to give 6 as a tan colored foam. Yield 26.3 g (83.13%). The spectral data of the product agreed with structural assignments. The compound was homogeneous by TLC and it was used in the next step without further purification.

9-(2,3,5-Tri-O-acetyl-ß-D-ribofuranosyl)-purine-6-thiocarboxamide 7: A solution of 6 (25.0 g, 0.062 mol) in pyridine (1000 ml) was cooled in an ice-salt bath for 30 minutes, and a slow stream of hydrogen sulfide gas was passed through it for 1 hour. The bath was removed and the reaction mixture was stirred at room temperature for 2 hours. TLC of the reaction mixture indicated the completion of the reaction. A steady stream of nitrogen gas was passed through the reaction mixture to drive off unreacted hydrogen sulfide, then the pyridine was evaporated under reduced pressure. The residue was co-evaporated with toluene (2 x 200 ml). The dark foam was then coated onto silica gel (70 g) and loaded onto a column packed with silica gel in chloroform. The column was eluted with chloroform and the fractions containing compounds with identical Rf values were combined. After evaporation 7 was obtained as an orange form. Yield 24.6 g (90.75%). Spectral data of the product were in agreement with reported values.

9-ß-D-Ribofuranosylpurine-6-thiocarboxamide 8: To a stirred solution of 7 (24.0 g, 0.055 mol) in methanol (700 ml) methanolic sodium methoxide solution (1N) was added to adjust the pH to 9. The reaction mixture was stirred at room temperature for 4.5 hours, at the end of which TLC showed the completion of the reaction. Glacial acetic acid was added to the reaction mixture to adjust the pH to 4. Charcoal (10.0 g) was added and the mixture was stirred at room temperature for an hour, then filtered through a celite bed. The celite bed was washed with methanol until the washings were colorless. The filtrate was concentrated to 200 ml and allowed to stand at room temperature overnight. The light orange, fine crystals were filtered to yield 9.5 g of 8. The mother liquor was concentrated and cooled to give an additional 3.2 g of the product. The combined yield was 12.7 g (74.2%), m.p. 169°C (Lit. m.p.-167-169°C).

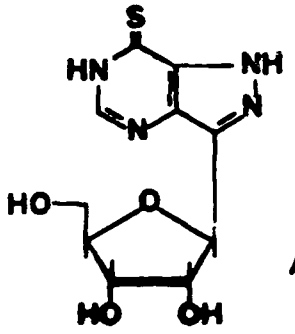
Following this procedure twice, a total of 21.0 g of final product was obtained. The spectral data of the combined batches were in agreement with reported values. Elemental analysis was within ± 0.4% of calculated values.

V. Discussion of Uncompleted Target Compounds

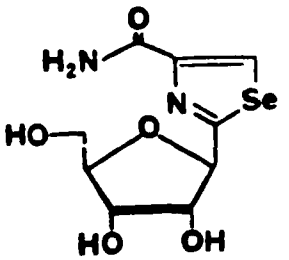
List of Compounds in Progress:

AVS 52	Thioformycin B
AVS 136	1-B-D-Ribofuranosyl-4-hydroxy-1,2,3-triazole-5 carboxamide
AVS 253	Selenazole
AVS PIP	4-Carboxamidine-4-phenylpiperidine dihydrochloride
AVS XYZ	Prodrug ester
AVS RCN	1-B-D-Ribofuranosyl-1,2,4-triazole-3-carbonitrile

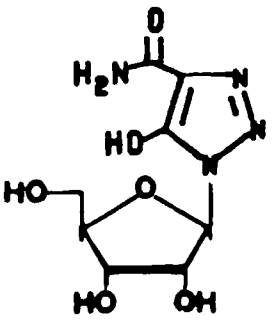
Structures of Compounds in Progress



AVS 52

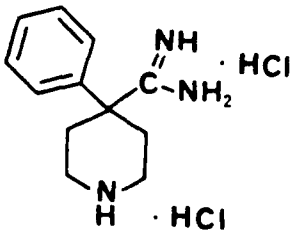


AVS 253

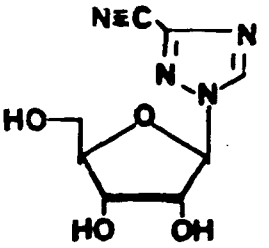


AVS 136

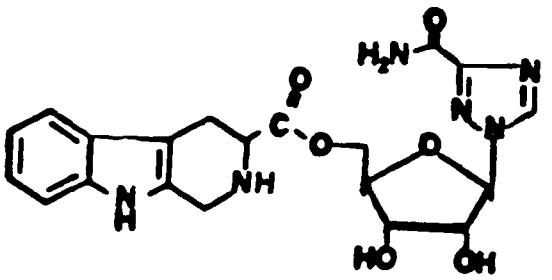
Structures of Compounds in Progress



AVS PIP



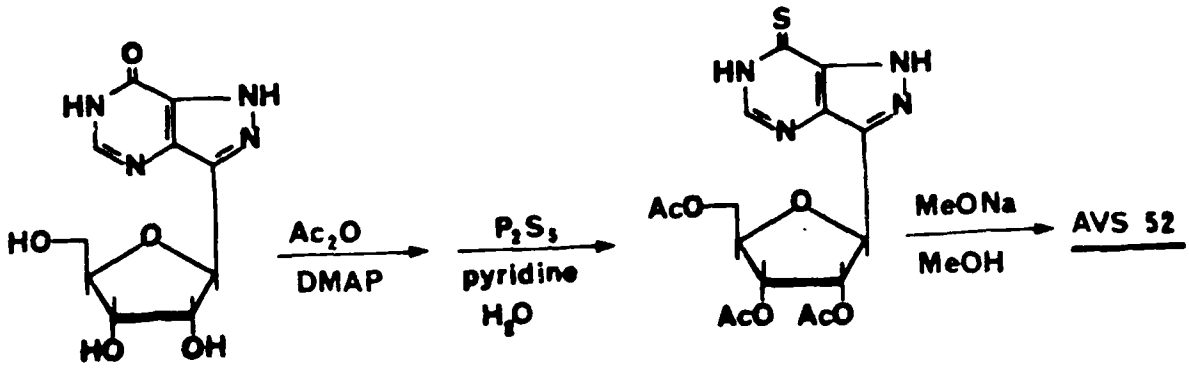
AVS RCN



AVS XYZ

22. Thioformycin B (AVS 52)

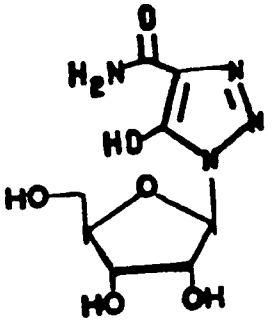
AVS 52 is presently being prepared according to the following scheme:[6]



Thioformycin B (8 g) has been acylated. The obtained protected nucleoside was purified, and presently the exchange of the purine oxygen by sulfur is being performed.

23. 1-B-D-Ribofuranosyl-4-hydroxy-1,2,3-triazole-5-carboxamide (AVS 136)

The synthesis of AVS-136 has been reported in the literature, however, the yield is expected to be very low. The project is presently on a delayed status to await the results of activity studies performed on its triazole hetero base (AVS-94).



AVS 136

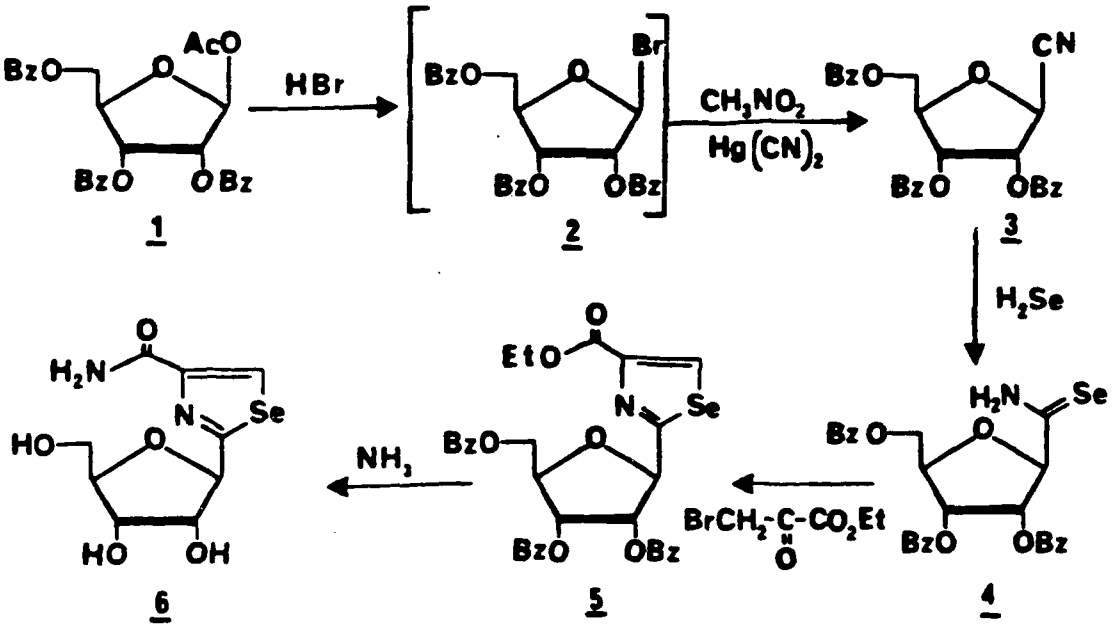
24. Selenazole (AVS 253)[7]

AVS-253 has previously been prepared according to the scheme shown. Presently, alternative methods are being evaluated to circumvent the direct use of extremely hazardous hydrogenselenide gas.

Synthetic Procedure

2-β-D-Ribofuranosylselenazo-4-carboxamide

AVS 253

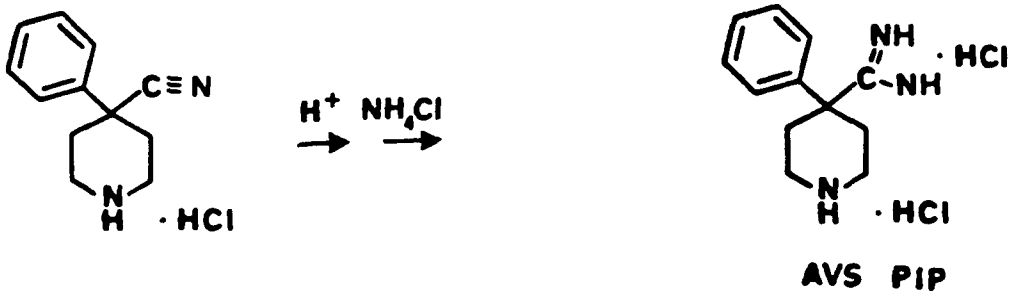


1 2 3 4 5 6

AVS 253

25. 4-Carborxamidine-4-phenylpiperidine dilhydrochloride (AVS PIP)[8]

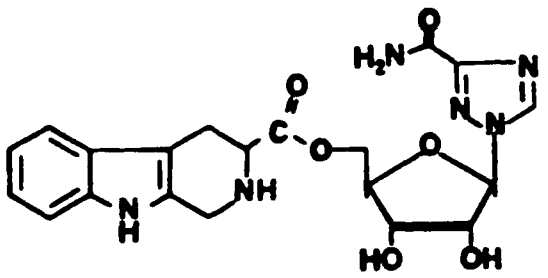
The amidination of 4-cyano-4-phenyl-piperidine as a model for amidinations under acidic conditions is presently being investigated. It can be expected that the obtained experimental parameters can be utilized in the transformation of related nitriles into amidine hydrochlorides without utilizing liquid ammonia/pressure procedures.

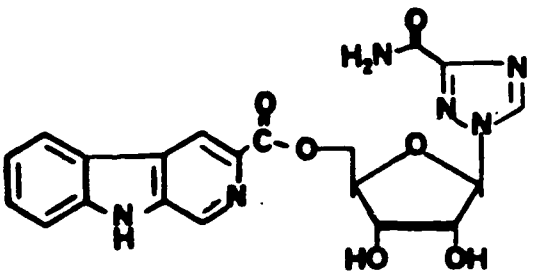


AVS PIP

26. Prodrug Ester (AVS XYZ)[9]

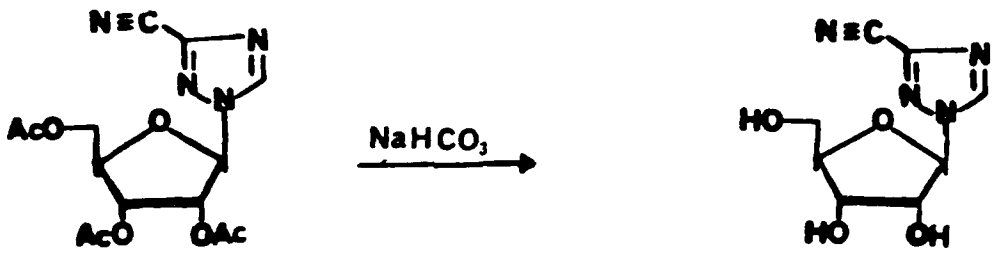
An adequate supply of β-carboline-3-carboxylic acid has been prepared and its coupling with ribavirin to form the ester linkage is under investigation. The obtained ester will be evaluated as a possible carrier system for drug delivery across the blood-brain barrier.





27. 1-B-D-Ribofuranosyl-1,2,4-triazole-3-carbonitrile (AVS RCN)

The tri-acetylated 1,2,4-triazole-3-carbonitrile nucleoside, obtained from acertyribavirin by dehydration, is the active intermediate in the preparation of ribavirin amidine hydrochloride (AVS-206). Presently, experimental procedures are modified to find a mild method that deblocks the nucleoside without altering the carbonitrile. Such a deblocked nucleoside carbonitrile is intended for the study of various amidination methods.



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VII. Acknowledgments

The personnel assigned to this contract during the past annual period were:

Ernst M. Schubert, Ph.D., Principal Investigator

Krishna Upadhya, Ph.D., Principal Assistant

Jay Dare, B.S., replaced by Valerie Nottingham, B.S., July 1988

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VIII. Appendix: List of Presentations of Scientific Meetings

a. Second International Conference on Antiviral Research.
Williamsburg, Virginia, April 10-14 1988.
"Anti-RNA-Viral Activities of Phenanthridones Related to Narciclasine." B. Gabrielsen, M.A. Ussery, P.G. Canonico, G.R. Pettit, E.M. Schubert, R.W. Sidwell. USAMRIID, Arizona State University, Pharm-Eco Laboratories and Utah State University.

b. Third Chemical Congress of North America.
Toronto, Canada, June 5-10, 1988.
"Antiviral Structure/Activity Study of the Phenanthridone Alkaloids: Pancretistatin. Narciclasine and Related Compounds." B. Gabrielsen, M.A. Ussery, P.G. Canonico, E.M. Schubert, G.R. Pettit, W.M. Shannon.

c. American Chemical Society 196th National Meeting.
Los Angeles, California, September 25-30, 1988.
"Preparation and Antiviral Evaluation of Deoxygenated Ribavirin and Tiazofurin Derivatives." K.G. Upadhya, J. Da Re, E.M. Schubert, B.J. Gabrielsen.

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